with those given ≥10 mg/kg/day of the mean ribavirin dose in this study [26.9% (49/182) *vs* 12.4% (26/209), $P < 0.001$] (data not shown). It seems possible to start ribavirin at a lower dose and increase it by degrees with monitoring of Hb level during treatment of patients with mild anaemia or ischemic heart disease, because the ribavirin dose appears to affect the viral relapse as the total dose over 48 weeks, not during the first 12 weeks.

In conclusion, our results have demonstrated that Peg-IFN α-2b is dose-dependently correlated with c-EVR and maintaining as high a drug dose of Peg-IFN α-2b as possible (≥1.2 μg/kg/week) during the first 12 weeks can yield higher c-EVR rates, leading to better treatment outcomes for patients with CH-C genotype 1.

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Impact of early viral kinetics on pegylated interferon alpha 2b plus ribavirin therapy in Japanese patients with genotype 2 chronic hepatitis C

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SUMMARY. The recommended therapy for genotype-2 chronic hepatitis C is a regimen of pegylated interferon alpha (peginterferon) plus ribavirin. This study was conducted to determine the value of early viral kinetics as a predictive factor for sustained virologic responder (SVR). Peginterferon alpha 2b (1.5 μg/kg/week) plus weight-based ribavirin (600–1000 mg/day) was administered to 51 patients with chronic HCV genotype 2 for 24 weeks. The HCV-RNA loads were measured at the baseline, hour 24, and week 1. The rebound index (RI, an index obtained from the viral load of week 1 divided by that of hour 24) was calculated. Compared with the baseline, the viral load at hour 24 for SVR was reduced by more than 1-log; it continued to decline thereafter, and at week 1 it was significantly lower than at hour 24 ($P < 0.05$). The viral load for non-SVR increased again between hour 24 and week 1. The SVR of patients with RI ≤1.0 was 100% (39/39). The SVR conversion for rapid virologic responders was 92% (35/38). The RI (≤1.0) was the only significant independent factor for SVR by multiple logistic regression analysis and is the first predictive factor in 24-week peginterferon plus ribavirin therapy for patients infected with genotype 2.

Keywords: chronic hepatitis C, early viral kinetics, genotype 2, pegylated interferon plus ribavirin, rebound index.

INTRODUCTION

The pegylated interferon alpha 2b (peginterferon) plus ribavirin combination therapy is recommended to treat genotype 2 chronic hepatitis C [1,2]. The two major predictive factors for a sustained virologic response (SVR) to interferon therapy are hepatitis C virus (HCV) genotype and viral load [3–7]. In Japan, the major genotypes include types 1 and 2 [8]. Compared with the former, the therapeutic efficacy of IFN is higher with the latter [8,9]. The duration of peginterferon plus ribavirin therapy for chronic hepatitis C is defined as 48 weeks for genotype 1 and 24 weeks for genotype 2 [10,11]. Attempts have been made to shorten the duration of the peginterferon plus ribavirin therapy for genotype 2 from 24 weeks to 12 or 16 weeks for rapid virologic responders (RVR; undetectable HCV-RNA at week 4) [12–18]. When peginterferon plus ribavirin is administered for 24 weeks, the rate of SVR is about 80% with relapse occurring in about 20%. It is believed that RVR is the primary predictive factor for SVR in the treatment of peginterferon plus ribavirin for genotype 2.

This study focused on early viral kinetics and RVR as predictive factors for SVR in the treatment of HCV patients with genotype 2 with peginterferon plus ribavirin. It was determined that rebound index (RI), a new index computed from early viral kinetics, is the first predictive factor for SVR and a substitute for RVR.

Abbreviations: EVR, early virologic response; NVR, nonvirologic response; RI, rebound index; RVR, rapid virologic response; SVR, sustained virologic responder.

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PATIENTS AND METHODS

Chronic HCV genotype 2 infected patients were eligible for enrollment if they fulfilled the following pretreatment criteria: baseline elevated serum alanine aminotransferase (ALT) levels, detectable serum HCV RNA via nucleic acid testing, HCV genotype 2, viral loads ≥5.30 log IU/mL, age ≥30 years, and a liver biopsy in the past 3 months consistent with chronic hepatitis (F1–F3) diagnosed based on the scoring system of Desmet *et al.* [19]. Fifty-one patients were treated with subcutaneous peginterferon alpha 2b (1.5 μg/kg/week) (PegIntron; Schering-Plough, Osaka, Japan) and

oral ribavirin (600 mg/day based on weight: <60 kg, 800 mg; 60–80 kg, 1000 mg; >80 kg) (Rebetol; Schering-Plough) for 24 weeks.

The 51 patients who participated in this study consisted of 28 males and 23 females ranging in age from 30 to 71 years, with a mean age of 52.1 years. Treatment was interrupted in three patients due to the development of adverse events. The remaining 48 patients completed 24 weeks of treatment. For all 48 patients, the total dosage of peginterferon or ribavirin exceeded 80% of the planned total dosage.

Peginterferon was administered at 9:00 in the morning at the initial, second, and third dosing points. The HCV loads were tested immediately before the start of treatment, at hour 24, and in weeks 1 and 2. The coefficient derived by dividing the viral load of week 1 by that of hour 24 was defined as the RI. The patients were grouped into the following 3 groups based on the RI and viral load in week 1: group A, RI >1.0; group B, RI ≤1.0 and viral load ≥3.70 log IU/mL in week 1; group C, RI ≤1.0 and viral load <3.70 log IU/mL in week 1.

The qualitative test for HCV-RNA was conducted five times (at weeks 4, 8, and 12, at the completion of treatment, and at week 24 after the completion of therapy). Patients showing the absence of HCV-RNA by week four were designated as RVR; and those with viral negativity between weeks 5 and 12, early virologic responders (EVR). The patients who remained HCV-RNA-negative until week 24 after the therapy was completed were defined as SVR and all other patients were designated non-SVR. Those who failed to achieve HCV-RNA negativity by the end of the treatment were designated nonvirologic responders (NVR).

Frozen sera were collected from the patients before and during IFN treatment, and the viral loads were measured by employing a quantitative HCV-RNA PCR assay (COBAS Amplicor HCV Monitor Test version 2.0 using a 10-fold dilution method, Roche Diagnosis, Tokyo, Japan), which has a lower threshold of quantification of 3.70 log IU/mL and an outer limit of quantification of 6.71 log IU/mL. A quantitative test for serum HCV-RNA was performed by using an Amplicor-HCV kit version 2.0 (Roche Diagnosis) and the results were labelled as positive or negative. The lower limit of detection was 1.70 log IU/mL. The preserved serum that produced a negative result for qualitative analysis of HCV-RNA was later re-examined by using the COBAS TaqMan HCV (AUTO) (Roche Diagnosis). If both tests produced negative results, the sample was judged to be HCV-RNA-negative. All testing was performed at a single reference laboratory. The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. The protocol for genotyping was carried out as previously described.

The criteria for exclusion were: (i) clinical or biochemical evidence of hepatic decomposition; and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment. Both peginterferon alpha-2b and ribavirin were discontinued if the haemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dL, 1000/mm^3 and 25 000/mm^3, respectively. The treatment was also discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia or severe haemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient no longer desired to continue treatment.

This study was conducted at the Shin-Kokura Hospital between December 2004 and June 2007. The study protocol was approved by the institutional ethics committee of Shin-Kokura Hospital and all patients gave informed consent to participate in this study, which was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Sustained virologic responder was analysed on an intention-to-treat basis. Differences between viral loads among groups were analysed using the Student's *t*-test and Mann–Whitney rank-sum test. Multivariate logistic regression analysis was used to determine predictive factors for SVR. We also calculated odds ratios and 95% confidence intervals. Predictive factors associated with SVR included: age, sex, body mass index (BMI), HCV-RNA loads, ALT levels, platelet counts, haemoglobin levels, RI, and the time HCV-RNA became undetectable.

All statistical analyses were conducted on a Macintosh computer using STATVIEW 5.0 (Abacus Concepts, Berkeley, CA, USA). Values for $P < 0.05$ were considered to be statistically significant.

RESULTS

Patient population

Of 51 patients, 48 patients completed the 24-week regimen. Of these 48 patients, 40 achieved SVR, resulting in an SVR rate of 83.3% (40/48). Seven patients remained HCV-RNA negative until week 24 of treatment but became positive again after the completion of the treatment. One patient failed to achieve HCV-RNA negativity by the end of the treatment. Of the three patients who interrupted treatment, two patients dropped out in weeks 17 and 19 due to general malaise and the other patient suffered from systemic eczema in week 5, necessitating the interruption of medication. Two of these three patients were SVR and one was non-SVR. The intention to treat analysis yielded a figure of 82.4% (42/51).

Table 1 Baseline characteristics of patients by SVR and non-SVR

	SVR $n = 42$	Non-SVR $n = 9$	Total $n = 51$
Age (years)	50.8 (12.7)	57.5 (12.7)	51.7 (12.6)
Male (%)	23 (55%)	5 (55%)	28 (55%)
Laboratory			
ALT (IU/L)	115 (111)*	56 (16)*	108 (106)
Haemoglobin (g/dL)	14.6 (1.6)	15.1 (1.3)	14.6 (1.5)
Platelet count ($\times 10^4/mm^3$)	19.1 (6.3)†	16.2 (6.2)†	18.7 (6.2)
HCV RNA loads (log IU/mL)	5.95 (0.47)‡	6.45 (0.33)‡	6.01 (0.48)
BMI (kg/m^2)	22.7 (2.8)	22.2 (3.6)	22.6 (2.8)

Values represent means with standard deviation in parentheses or as absolute values with percentages in parentheses.
*$P < 0.01$ for SVR *vs* non-SVR.
†$P < 0.001$ for SVR *vs* non-SVR.
‡$P < 0.05$ for SVR *vs* non-SVR.
SVR, sustained virologic responder; ALT, alanine aminotransferase; BMI, body mass index.

The baseline characteristics of these 51 patients by SVR and non-SVR are shown in Table 1. The mean age was not significantly different between SVR at 50.8 years and non-SVR at 57.4 years. The ALT level and platelet counts were significantly higher ($P < 0.01$ and $P < 0.001$, respectively) while the HCV-RNA load was significantly lower ($P < 0.05$) in patients with SVR.

Early viral kinetics and rebound index in relation to SVR and non-SVR

Early viral kinetics and the RI in relation to SVR and non-SVR are shown in Table 2 and Fig. 1. HCV-RNA load for all SVR patients was reduced by 1-log in hour 24. The viral load thereafter (in week 1) was significantly reduced in contrast to that of hour 24 ($P < 0.05$). Furthermore, in week 2, the viral load was significantly reduced compared with that of week 1 ($P < 0.001$). With the exception of one patient, none of the patients with SVR showed a rise in the viral load in week 1. Compared with the baseline, the viral load in week 1 was reduced by more than 1-log in all SVR patients. The viral load for non-SVR was reduced by 1-log in hour 24 but a 1-log reduction was not achieved with NVR. Thereafter, the viral load rose again in week 1, then was reduced in week 2. The viral loads of all nine patients exhibited an increase in week 1, and the viral load of three of these patients in week 1 failed to be reduced from the baseline by 1-log. Among these three patients, HCV-RNA became negative in week 12 in two patients but reverted to positive 1 month after the completion of the treatment. The RI

Table 2 Kinetics of HCV RNA and RI during the first 2 weeks of treatment relative to SVR and non-SVR.

	SVR ($n = 42$)	Non-SVR ($n = 9$)	Total ($n = 51$)
HCV loads (log IU/mL)			
Before	5.95 (0.47)*	6.45 (0.33)*	6.01 (0.48)
Hour 24	4.56 (0.75)†	4.97 (1.23)	4.68 (0.86)
Week 1	4.02 (0.69)†,‡	5.49 (0.90)	4.30 (0.95)
Week 2	3.77 (0.45)‡	4.14 (1.15)	3.97 (0.85)
RI	0.63 (0.09)*	2.13 (0.33)*	0.92 (0.12)

Values represent means with standard deviation in parentheses. SVR, sustained virologic responder; SD, standard deviation.
*$P < 0.05$ for SVR *vs* non-SVR.
†$P < 0.05$ for hour 24 *vs* week 1 in SVR.
‡$P < 0.001$ for week 1 *vs* week 2 in SVR.

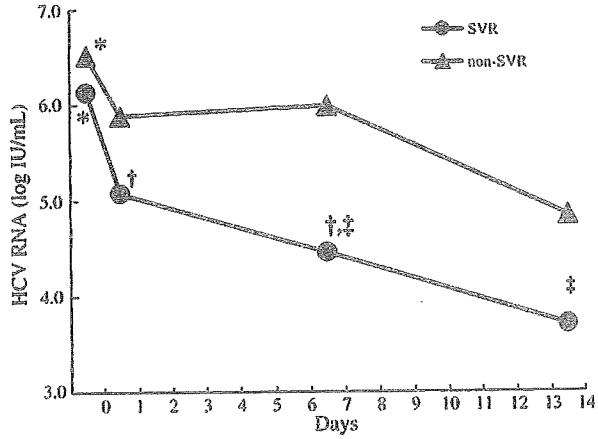

Fig. 1 Kinetics of HCV-RNA during the first 2 weeks of therapy relative to SVR (black circle) and non-SVR (black triangle). *$P < 0.05$ for SVR *vs* non-SVR, †$P < 0.05$ for hour 24 *vs* week 1 in SVR, ‡$P < 0.001$ for week 1 *vs* week 2 in SVR. SVR, sustained virologic response.

(0.43) of SVR patients was significantly lower than that of non-SVR (4.13) ($P < 0.05$).

SVR and non-SVR in relation to the timing of HCV-RNA negativity in groups A, B and C

Sustained virologic responder and non-SVR for groups A, B and C stratified by the timing of HCV-RNA negativity are shown in Table 3. Among the 48 patients, there were 38 RVR (79.2%), 9 EVR (18.7%), and 1 NVR (2.1%). The percentages for achieving SVR by RVR, EVR, and NVR were 92.1%, 55.6%, and 0.0%, respectively. The percentages of achieving SVR in groups A, B and C were 11%, 100%, and 100%, respectively. In groups B and C with the

Table 3 SVR and non-SVR in relation to the timing of HCV-RNA negativity in group A, B and C

	RVR $n = 38$	EVR $n = 9$	NVR $n = 1$	Total $n = 48$
RI>1.0 (Group A, $n = 9$)				
SVR (%)	1 (25)	0 (0)	0 (0)	1 (11)
Non-SVR (%)	3 (75)	4 (100)	1 (100)	8 (89)
RI ≤1.0, ≥3.7 log IU/mL* (Group B, $n = 23$)				
SVR (%)	18 (100)	5 (100)	0 (0)	23 (100)
Non-SVR (%)	0 (0)	0 (0)	0 (0)	0 (0)
RI ≤1.0, <3.7 log IU/mL* (Group C, $n = 16$)				
SVR (%)	16 (100)	0 (0)	0 (0)	16 (100)
Non-SVR (%)	0 (0)	0 (0)	0 (0)	0 (0)
Total ($n = 48$)				
SVR (%)	35 (92)	5 (56)	0 (0)	40 (83)
Non-SVR (%)	3 (8)	4 (44)	1 (100)	8 (17)

*HCV RNA loads at week 1.
SVR, sustained virologic responder; RVR, rapid virologic responder; EVR, early virologic responder; NVR, non-virologic responder; RI, rebound index.

RI below 1.0, all became HCV-RNA-negative within 8 weeks, thus achieving SVR status. The 24-week peginterferon plus ribavirin treatment for genotype 2 required the RI to be less than 1.0. Among the patients with a RI of ≤1.0, 16 had a viral load of less than 3.7 log IU/mL (Group C) at week 1. These patients were considered to be super-high responders to peginterferon. The early viral kinetics of these patients are shown in Table 4. The group included nine males and seven females with a mean age of 47.1 years. The mean age for men was 50.6 years, which was higher than 42.8 years for women but the difference was not statistically significant. The viral load of these 16 patients before treatment was 5.90 log IU/mL, which was significantly lower than 6.22 log IU/mL, viral load for other SVR ($P < 0.01$). Of these 16, the viral load up to hour 24 was less than 3.70 log IU/mL in six patients. HCV-RNA was negative in week 2 in five of these six patients.

The viral loads of SVR patients in group A at baseline (RI >1.0), hour 24, weeks 1 and 2 were 6.08, 3.95, 4.40, and <3.70 log IU/mL, respectively. Compared with the viral load immediately before treatment, that at hour 24 was reduced by more than 2-log10.

Three patients interrupted treatment

Among the 51 patients who participated in the study, treatment was interrupted in three due to the development of adverse effects. These patients dropped out in weeks 5, 17, and 19. In these three patients, HCV-RNA became negative in week 4 and their RI was below 1.0. The patient who was discontinued in week 5 showed a relapse of HCV-RNA during a subsequent observation. The viral load for the two patients who dropped out in weeks 17 and 19 was less than 3.70 log IU/mL in week 1, and HCV-RNA continued to be negative 24 weeks after drug withdrawal. These two patients were judged to be SVR.

Predictive factors of SVR by multivariate analysis

Rebound index (≤1.0) was the only significant independent factor for SVR by multiple logistic regression analysis (Table 5). All other factors were not significant.

Table 4 Early viral kinetics of patients in super-high responder group (Group C)

			HCV loads (log IU/mL)			HCV-RNA	
Number	Age	Sex	Before	Hour 24	Week 1	Week 2	Week4
1	30	M	6.23	<3.70	<3.70	Negative	Negative
2	42	M	5.37	<3.70	<3.70	Negative	Negative
3	45	M	5.98	<3.70	<3.70	Negative	Negative
4	56	M	6.20	<3.70	<3.70	Negative	Negative
5	32	F	5.40	<3.70	<3.70	Negative	Negative
6	66	F	5.54	<3.70	<3.70	Positive	Negative
7	42	M	5.41	4.64	<3.70		Negative
8	48	M	6.08	4.28	<3.70		Negative
9	59	M	6.08	4.46	<3.70		Negative
10	66	M	6.28	4.23	<3.70		Negative
11	67	M	5.70	4.80	<3.70		Negative
12	30	F	6.18	4.41	<3.70		Negative
13	31	F	5.89	4.51	<3.70		Negative
14	32	F	5.94	4.08	<3.70		Negative
15	42	F	5.40	4.34	<3.70		Negative
16	67	F	5.70	4.63	<3.70		Negative

Table 5 Predictive factors of SVR by multivariate analysis

Factor	Category	Odds ratio	95% CI	*P*-value
Age	≥50 years	0.622	0.035–11.114	0.746
	<50 years	1		
Sex	Male	1.972	0.109–35.799	0.646
	Female	1		
BMI	<22.5	1.251	0.085–18.462	0.871
	≥22.5	1		
HCV load	<6.0 logIU/mL	0.98	0.061–15.788	0.988
	≥6.0 logIU/mL	1		
ALT	<50 IU/L	0.757	0.038–15.240	0.856
	≥50 IU/L	1		
Platelet count	$\geq 18 \times 10^4/mm^3$	1.795	0.104–31.019	0.687
	$<18 \times 10^4/mm^3$	1		
Haemoglobin level	<14 mg/dL	0.398	0.012–12.7171	0.602
	≥14 mg/dL	1		
RI	≤1.0	689.586	4.214–>999.999	0.012
	>1.0	1		
Time to HCV RNA negativity(–)	≤Week 4	1.612	0.050–51.632	0.787
	>Week4	1		

BMI, body mass index; ALT, alanine aminotransferase.

DISCUSSION

The early viral kinetics in association with the peginterferon plus ribavirin treatment for genotype 1 have been reported [20,21]; but reports on early viral kinetics are scarce when the same combination is applied to genotype 2. This is the first investigation of early viral kinetics during peginterferon plus ribavirin therapy for genotype 2 chronic hepatitis C patients with high viral loads. We found that the RI (a new index) that is computed from the early viral kinetics is the first predictive factor for SVR as a substitute for RVR as a result of multiple analysis data. Patients with a RI of less than 1.0 and a viral load of less than 3.7 log IU/mL in week 1 were also identified as super-high responders to peginterferon plus ribavirin therapy.

The serum concentration of peginterferon alpha 2b peaked around 24 h, followed by a gradual decrease thereafter [22,23]. Thus the earlier studies on viral kinetics in association with peginterferon plus ribavirin for genotype 1 reported that the HCV load declines in hour 24 and increases again in week 1 [20,21]. In the responder group, the HCV load continues to decline every week thereafter [21]. A similar pattern is also seen in peginterferon monotherapy [22]. However, there are few reports on early viral kinetics involving genotype 2. In this study, the early viral kinetics of genotype 2 was investigated. Noting this increase in week 1, the viral load in week 1 was divided by that of hour 24 and the resultant coefficient was defined as the RI. In this study, the SVR rate was 100% for groups B and C, with the RI being less than 1.0. In these groups, HCV-RNA was eliminated by week 12 in all patients. On the other hand, re-emergence of virus was noted in 8% among the RVR. These findings suggested that the RI is the first predictive factor for SVR as a substitute for RVR in 24-week peginterferon plus ribavirin therapy for genotype 2. For those patients with a RI of >1.0, treatment lasting more than 24 weeks appeared necessary.

Peginterferon plus ribavirin therapy results in SVR exceeding 80% in genotype 2 patients when treatment lasts for 24 weeks [1,2,9]. Because these patients are high responders to peginterferon plus ribavirin therapy, attempts have been made to shorten the duration of treatment [12–18]. Earlier, RVR patients have been treated for shorter periods (e.g. 12, 14 and 16 weeks) and it was reported that there was no difference in the SVR rate compared with the treatment duration of 24 weeks [12–15] According to a recent randomized study, the SVR rate is high even in RVR patients when treated for 24 weeks [16]. It has been reported that shortening of the treatment period results in economic advantages and reductions in the development of side effects [17]. Thus it becomes necessary to evaluate the super-high responder group to peginterferon plus ribavirin therapy who do not show reductions in the SVR rate even when the duration of treatment is reduced. Among those with genotype 2, about 80% or more convert to RVR but RVR alone does not sufficiently explain the state of super-high responders. In interferon therapy of genotype 2 patients, peginterferon alone produces therapeutic effects [2]. An HCV load below 3.0 log IU/mL on day 7 and undetectable HCV-RNA on day 29 were predictive of successful short-term treatment [18]. It is essential to identify super-high responders to peginterferon by using the early viral kinetics during the first 2 weeks of therapy. In this study, the viral load was investigated in week 1 following the

start of therapy, probably before the therapeutic effect of ribavirin manifests. Among 38 patients with RVR, 16 (group C) were found to have a viral load of less than 3.7 log IU/mL in week 1. It was believed that these patients constitute super-high responders to peginterferon; and that a high SVR rate may be reached when the duration of the peginterferon plus ribavirin therapy is curtailed to less than 12 weeks. The viral load of the two patients who had discontinued treatment in weeks 17 and 19 was less than 3.7 log IU/mL in week 1, and both converted to SVR after discontinuation of treatment. Further studies on a larger scale are needed.

CONCLUSION

Rapid virologic responder is a predictive factor for SVR in peginterferon plus ribavirin therapy. However, it was proven that the RI that was computed from the early viral kinetics in this study is the first predictive factor for SVR as a substitute for RVR by multiple logistic regression analysis. Patients with a RI less than 1.0 and a viral load of less than 3.7 log IU/mL (below the detectable level) in week 1 are also considered to be super-high responders to peginterferon plus ribavirin, thus constituting a group for whom the treatment period may be shortened. Further studies on a larger scale are necessary.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Effective prediction of outcome of combination therapy with pegylated interferon alpha 2b plus ribavirin in Japanese patients with genotype-1 chronic hepatitis C using early viral kinetics and new indices

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Abstract
Background The rates of sustained virologic response (SVR) and relapse with pegylated interferon alpha 2b (peginterferon) plus ribavirin in patients with genotype-1 chronic hepatitis C (CHC) are approximately 50 and 30%, respectively. We investigated whether SVR and transient response (TR) can be differentiated during treatment using new indices calculated from early viral kinetics and the timing of when hepatitis C virus (HCV)-RNA becomes undetectable.
Methods Peginterferon alpha 2b (1.5 μg/kg per week) plus weight-based ribavirin (600–1,000 mg/day) were administered to 141 patients with genotype-1 CHC for 48 weeks. The HCV-RNA loads were measured at baseline, 24 h, week 1, and week 2. The rebound index (RI, viral load at week 1 divided by viral load at 24 h) and the second rebound index (RI-2nd, viral load at week 2 divided by viral load at 24 h) were calculated.
Results With SVR, the viral load was reduced at 24 h, did not rise during week 1 (RI $\leq$ 1.0), and was significantly reduced at week 2 ($P < 0.05$). Viral loads with TR and non-response increased at week 1. The SVR rate was 90% with RI $\leq$ 1.0, 96% with rapid viral responders, and 93% with RI-2nd <0.7 and week 8 early viral responders. The SVR rate with these 3 groups was 90% and administration for 48 weeks was recommended. With other groups, the SVR rate was 23% and the TR rate was 77%. Administration for 72 weeks was therefore recommended.
Conclusions We distinguished SVR from TR during treatment using two indices (RI and RI-2nd) and the timing of HCV-RNA negativity.

Keywords Chronic hepatitis C · Pegylated interferon plus ribavirin · Early viral kinetics · Rebound index · Genotype 1

Abbreviations

SVR	Sustained virologic response
TR	Transient response
NR	Non-response
RI	Rebound index
RI-2nd	Second rebound index
RVR	Rapid viral responder
W8EVR	Week 8 early viral response
W12EVR	Week 12 early viral response
LVR	Late viral responder
NVR	Non-viral responder

Introduction

The first choice of treatment of genotype 1 chronic hepatitis C (CHC) is combination therapy with pegylated interferon alpha 2b (peginterferon) and ribavirin. The duration of treatment for genotype 1 is 48 weeks [1, 2]. Factors predictive of sustained virologic response (SVR) to

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peginterferon plus ribavirin include genotype, viral load, age, histology, and amino acid substitutions in the hepatitis C virus (HCV) [3–6]. None of these predictive factors is adequate in predicting SVR in patients with genotype 1 and a high viral load prior to treatment.

With the current standard of care of peginterferon plus ribavirin administered for 48 weeks, the SVR rate in patients with genotype 1 and a high viral load is about 50% [1, 2]. It has been recognized, however, that the SVR rate increases if the duration of treatment is extended to 72 weeks [7–9]. The result of treatment with peginterferon plus ribavirin is SVR, transient response (TR), or non-response (NR). At the end of treatment, about 80% of patients are HCV RNA-negative, but about 30% of these patients relapse (TR) after the end of treatment, resulting in an actual SVR rate of about 50%. To increase the SVR rate, this incidence of relapse must be reduced; to achieve this, the duration of treatment needs to be extended to 72 weeks for patients who may potentially relapse after the end of treatment [7]. Differentiation between SVR and TR is therefore essential during treatment.

HCV RNA negativity status at weeks 4 and 12 during treatment is important in predicting SVR [6, 10–12], with reduction in the SVR rate observed if HCV RNA is not undetectable by week 12. In other words, for the early determination of the therapeutic efficacy of peginterferon plus ribavirin treatment, HCV RNA negativity by week 4 (rapid viral responder: RVR), HCV RNA negativity by week 12 (early viral responder: EVR), and HCV RNA negativity by week 24 (late viral responder: LVR) are considered important. EVR is a better predictor of SVR than the predictive factors that can be determined prior to treatment. EVR is therefore considered to be an index of therapeutic effect in the early stage of peginterferon plus ribavirin treatment. In a recent trend, a duration of treatment of 72 weeks is being selected when HCV RNA is detected at week 12 but is undetectable at week 24. However, distinguishing SVR from TR during treatment is difficult by these two time points when HCV RNA is not detected.

For a more accurate determination of SVR and TR during treatment, HCV RNA was examined at week 8 in addition to weeks 4, 12, and 24, and the SVR rate was examined based on HCV RNA negativity at these time points. Early viral kinetics up to week 2, considered to be the index of the therapeutic effect of peginterferon alone, were also evaluated and two new indices were defined. Distinguishing SVR from TR during peginterferon treatment was possible by combining these new indices and the timing of HCV RNA negativity and allowed the assignment of patients to 48- or 72-week treatment as a result.

Patients and methods

A total of 149 patients with genotype 1 CHC were treated with peginterferon plus ribavirin at the Shin-Kokura Hospital between December 2004 and May 2006. Of these patients, treatment was interrupted in 8 patients, so this study was conducted on the remaining 141 patients who completed 48 weeks of treatment. Eighty were male and 61 were female, with ages ranging from 27 to 70 years (mean: 53.2 ± 10.8), and 109 individuals were naïve to interferon therapy. The viral load at enrollment exceeded 100,000 IU/ml. The results of liver biopsy conducted within 6 months of enrollment confirmed chronic hepatitis (F1-F3), and diagnosis was based on the scoring system of Desmet et al. [13]. All patients received 1.5 μg/kg of peginterferon alpha-2b (PegIntron, Schering-Plough, Osaka, Japan) administered subcutaneously once a week in combination with ribavirin (Rebetol, Schering-Plough, Osaka, Japan) administered orally at a daily dose of 600–1,000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60–80 kg, and 1,000 mg for those weighing more than 80 kg).

Peginterferon was administered at 9:00 in the morning for the initial, second, and third doses. The HCV loads were measured immediately before the start of treatment, at 24 h post-dose, and at weeks 1 and 2. The coefficient derived by dividing the viral load at week 1 by that at 24 h was defined as the rebound index (RI), while the coefficient derived by dividing the viral load at week 2 by that at 24 h was called the second rebound index (RI-2nd). The patients were divided into the following 3 groups based on RI and RI-2nd: RI-A group (RI $\leq$ 1.0), RI-B group (RI $>$ 1.0 and RI-2nd $<$ 0.7), and RI-C group (RI $>$ 1.0 and RI-2nd $\geq$ 0.7).

The qualitative test for HCV RNA was conducted 6 times (at weeks 4, 8, 12, and 24, at the end of treatment, and at week 24 after the end of treatment). Patients who were HCV RNA-negative by week 4 were considered rapid viral responders (RVR), patients who were HCV RNA-negative between weeks 5 and 12 were considered early viral responders (EVR), and patients HCV RNA-negative between weeks 13 and 24 were considered late viral responders (LVR). EVR was further divided into week 8 EVR (HCV RNA-negative between weeks 5 and 8, W8EVR) and week 12 EVR (HCV RNA-negative between weeks 9 and 12, W12EVR). Patients HCV RNA-positive at week 24 were considered non-viral responders (NVR). Patients who remained HCV RNA-negative up to 24 weeks after the end of treatment were considered to have achieved SVR. Patients HCV RNA-negative by week 24 of treatment but who became positive again after the end of treatment were considered TR. Patients who failed to achieve HCV RNA negativity by the end of treatment were

considered NR. None of these patients were HCV RNA-negative between weeks 25 and 48.

Sera were collected from the patients before and during treatment and frozen for determination of viral loads by a quantitative HCV RNA PCR assay (COBAS Amplicor HCV Monitor Test v2.0 using a 10-fold dilution method, Roche Diagnostics, Tokyo, Japan), which has a low threshold of quantitation of 5,000 IU/ml and an outer limit of quantitation of 5,100,000 IU/ml. A qualitative test for serum HCV RNA was performed using Amplicor-HCV kit version 2.0 (Roche Diagnostics, Tokyo, Japan) and the results were labeled positive or negative. The lower limit of detection was 50 IU/ml. All testing was performed at a single reference laboratory. The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. Genotyping was carried out as described previously [14].

Criteria for exclusion were [1] clinical or biochemical evidence of hepatic decompensation and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma [2], white blood cell count of less than 3,000/mm^3 and platelet count of less than 50,000/mm^3 [3], concurrent liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive), [4] excessive active alcohol consumption over 60 g/day or drug abuse, [5] severe psychiatric disease, or [6] antiviral or corticosteroid therapy within the 12 months prior to enrollment. Both peginterferon alpha-2b and ribavirin were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dl, 1,000/mm^3 and 25,000/mm^3, respectively. Treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, if continuation of treatment was judged not to be possible by the attending physician, or if the patient no longer desired to continue treatment.

Informed consent

The study protocol was approved by the Institutional Ethics Committee of Shin-Kokura Hospital, and all patients gave informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Statistical analysis

Differences between viral loads between two groups were analyzed using the Student's t test and Mann–Whitney rank-sum test. We conducted analysis using the Kruskal Wallis test for three-group (SVR, TR, and NR) and five-group (RVR, W8EVR, W12EVR, LVR and NVR) comparisons. All statistical analyses were conducted on a Macintosh computer using StatView 5.0 (Abacus Concepts, Berkeley, CA, USA). P values of <0.05 were considered to be statistically significant.

Results

Baseline characteristics of patients grouped by SVR, TR and NR

SVR was observed in 72 patients (51.1%), TR in 40 patients (28.4%), and NR in 29 patients (20.6%). The characteristics at enrollment of patients showing SVR, TR, or NR are presented in Table 1. The mean age of SVR, TR, and NR patients was 50.8, 56.6, and 56.0 years, respectively. There were no significant inter-group differences in mean age, gender, or pre-treatment test results (alanine aminotransferase, hemoglobin level, platelet count, and viral load).

Table 1 Baseline characteristics of patients by response (SVR, TR, and NR)

	SVR $n = 72$	TR $n = 40$	NR $n = 29$	Total $n = 141$	P value[a]
Age (years)	50.8 (11.3)	56.6 (8.6)	56 (10.4)	53.2 (10.8)	0.084
Male (%)	41 (56)	24 (52)	19 (65)	84 (57)	
Laboratory					
ALT (IU/l)	88 (82)	86 (53)	94 (78)	90 (74)	0.808
Hemoglobin level (g/dl)	14.5 (1.4)	14.6 (1.3)	14.6 (1.0)	14.5 (1.3)	0.917
Platelet count ($\times 10^4$/mm^3)	19 (6)	17 (6)	20 (10)	19 (7)	0.707
HCV RNA loads ($\times 10^3$ IU/ml)	2299 (1634)	2228 (1344)	2390 (1501)	2298 (1524)	0.953
Body mass index (kg/m^2)	23.6 (5.7)	24.8 (2.9)	23.9 (3.6)	24 (4.9)	0.536

Values are represented as means with standard deviation in parentheses or as absolute values with percentages in parentheses

SVR sustained virologic response, *TR* transient response, *NR* non response, *ALT* alanine aminotransferase

[a] Kruskal Wallis Test

Early viral kinetics, RI and RI-2nd relative to SVR, TR and NR

Viral kinetics up to the first two weeks after the start of treatment are shown for the SVR, TR, and NR groups (Fig. 1 and Table 2). The viral load at 24 h for the SVR and TR groups (226,000 and 229,000 IU/ml) was reduced significantly compared to the NR group (523,000 IU/ml) ($P \leq 0.05$). The differences of the viral loads for three groups at weeks 1 and 2 were significant ($P \leq 0.0001$). The viral load of the SVR group at weeks 1 and 2 was significantly lower than that of the TR group ($P < 0.01$ and $P < 0.05$). The former was reduced at week 1 with no increases thereafter, and the viral load at week 2 (76,000 IU/ml) was significantly lower than at 24 h (226,000 IU/ml, $P < 0.01$). The viral load of the TR group rose to 397,000 IU/ml at week 1 and was reduced to 169,000 IU/ml at week 2. This reduction was not significant when compared against that at 24 h (229,000 IU/ml). The viral load of the NR group rose again to 1,206,000 IU/ml at week 1 and was reduced to 615,000 IU/ml at week 2, which was still higher than that at 24 h (523,000 IU/ml).

RI and RI-2nd for the SVR, TR and NR groups are shown in Table 2. RI for the SVR group (0.8) was below 1.0. The differences of the RI for the three groups were significant ($P \leq 0.0001$). RI-2nd for the SVR, TR and NR groups was 0.3, 0.8, and 1.3, respectively, with the highest value observed with the NR group. The differences of the RI-2nd for three groups were significant ($P \leq 0.0001$). RI-2nd for the TR group was significantly higher than for the SVR group ($P < 0.05$).

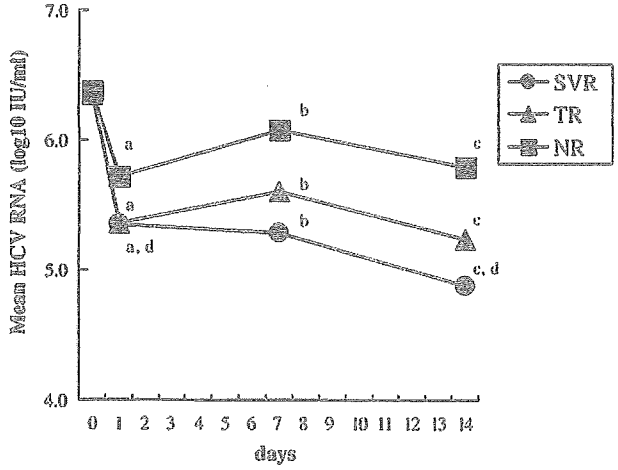

Fig. 1 HCV-RNA kinetics during the first 2 weeks of treatment by SVR (*black circle*), TR (*black triangle*), and NR (*black square*). a $P < 0.05$, b $P < 0.0001$, c $P < 0.0001$ (Kruskal Wallis test), d $P < 0.01$ (hour 24 vs. week 2 in SVR). *SVR* sustained virologic response, *TR* transient response, *NR* non-response

SVR rates, early viral kinetics, RI and RI-2nd relative to the timing of HCV RNA negativity

RVR, W8EVR, W12EVR, LVR, and NVR were observed in 26 (18.4%), 31 (22.0%), 31 (22.0%), 24 (17.0%), and 29 (20.6%), respectively. The SVR rate with the RVR, W8EVR, W12EVR, and LVR groups was 96.2% (25/26), 83.9% (26/31), 54.8% (17/31), and 16.7% (4/24), respectively. None in the NVR group exhibited the absence of HCV-RNA at the end of treatment. The HCV RNA kinetics for the RVR, W8EVR, W12EVR, LVR, and NVR groups up to week 2 of treatment are shown in Fig. 2 and Table 3. The viral load of the RVR group was rapidly reduced to 143,000 IU/ml by 24 h, with a further drop to 55,000 IU/ml at week 1. At week 2, the viral load was reduced to 8,000 IU/ml, which was significantly less than that at 24 h ($P < 0.001$). The viral loads for the W8EVR and W12EVR groups were reduced to 186,000 IU/ml and 134,000 IU/ml, respectively, by 24 h but rose to 231,000 IU/ml and

Table 2 Kinetics of HCV RNA during the first 2 weeks of treatment by response (SVR, TR, and NR)

	SVR ($n = 72$)		TR ($n = 40$)		NR ($n = 29$)		P value[a]
	Mean	SD	Mean	SD	Mean	SD	
HCV loads (×1000 IU/ml)							
Before treatment	2299	(1634)[b]	2228	(1344)	2390	(1301)	0.9538
Hour 24	226	(328)	229	(249)	523	(518)	0.0102
Week 1	190	(302)	397	(399)	1206	(811)	<0.0001
Week 2	76	(193)[b]	169	(249)	615	(617)	<0.0001
Rebound index	0.8	(0.9)	2.9	(3.1)	3.1	(1.8)	<0.0001
Rebound index 2	0.3	(0.6)	0.8	(0.7)	1.3	(1.1)	<0.0001

Values represent means with ranges in parentheses

[a] Kruskal Wallis Test

[b] $P < 0.01$ (hour 24 vs. week 2 in SVR)

Abbreviations *SVR* sustained virologic response, *TR* transient response, *NR* non response, *SD* standard deviation

277,000 IU/ml, respectively, at week 1. The viral load of the W8EVR group at week 2 was reduced to 49,000 IU/ml, which was significantly less than that at 24 h ($P < 0.001$). The viral load of the W12EVR group, on the other hand, was 119,000 IU/ml, which was not significantly less than that at 24 h. The viral load of the LVR group at 24 h (563,000 IU/ml) was higher than that of RVR, W8EVR, or W12EVR. The viral load rose further to 674,000 IU/ml at week 1, and although a reduction to 361,000 IU/ml was observed at week 2, it was still significantly greater than with RVR, W8EVR, and W12EVR ($P < 0.001$, $P < 0.001$, and $P < 0.01$, respectively). The viral load (523,000 IU/ml) of the NVR group at 24 h was similar to that of the LVR group. It rose after one week and was not low in the second week (615,000 IU/ml) compared to that at 24 h. The differences of the viral loads for five groups were significant at hour 24, week 1, or week 2 ($P \leq 0.0001$).

RI and RI-2nd of the RVR, W8EVR, W12EVR, LVR, and NVR groups are shown in Table 3. RI of RVR (0.4) was the lowest and was lower than that of W8EVR (2.3), W12EVR (2.4), LVR (1.5), and NVR (3.1). RI-2nd of NVR (1.3) was the highest, being higher than RVR (0.1), W8EVR (0.5), W12EVR (0.7), and LVR (0.7) ($P < 0.001$, $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively). The mean RI-2nd for other than NVR was below 0.7. The differences of the RI or RI-2nd for five groups were significant ($P \leq 0.0001$).

SVR, TR, and NR rates relative to RI and RI-2nd

The 3 groups (RI-A, RI-B, and RI-C) and 4 groups (RVR, W8EVR, W12EVR, and LVR) were combined and then divided into 12 groups, with SVR, TR, and NR grouped by

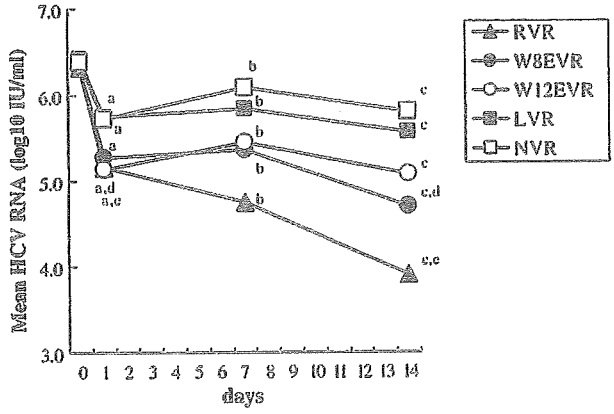

Fig. 2 HCV RNA kinetics by RVR (*black triangle*), W8EVR (*black circle*), W12EVR (*white circle*), LVR (*black square*), and NVR (*white square*) during the first 2 weeks of treatment a $P < 0.001$, b $P < 0.0001$, c $P < 0.0001$ (Kruskal Wallis test), d $P < 0.001$ (hour 24 vs. week 2 in W8EVR), e $P < 0.001$ (hour 24 vs. week 2 in RVR). *RVR* rapid viral response, *W8EVR*, week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non-viral response

RI and RI-2 and by RVR, W8EVR, W12EVR, and LVR (Fig. 3). The SVR, TR, and NR rates were 90.2% (46/51), 9.8% (5/51), and 0% (0/51), respectively, with RI-A (RI $\leq$ 1.0), 55.6% (25/45), 40.0% (18/45), and 4.4% (2/45), respectively, with RI-B (RI $>$ 1.0, RI-2nd $<$ 0.7), and 2.2% (1/45), 37.8% (17/45), and 60.0% (27/45), respectively, with RI-C (RI $>$ 1.0, RI-2nd $\geq$ 0.7). The SVR rate for RI-A and RVR was 90.2% (46/51) and 96.2% (25/26), respectively. The SVR rate for RI-B and W8EVR was 93.3% (14/15). The SVR rate for the patients in these 3 areas was 89.7% (61/68), suggesting that they represent the population for which 48-week treatment is appropriate. Among the 112 patients who became HCV RNA-negative at week 24, 60.7% (68/112) were in the above 48-week regimen area. In particular, the SVR rate (2.2%) from RI-C was very low and the TR and NR rates were 37.8% and 60.0%, respectively. Among W8EVR, W12EVR, and LVR in the range outside that for a 48-week regimen, the SVR rate (25.0%, 11/44) was low but the TR rate (75.0%, 33/44) was high. Thus extension of the treatment period was considered necessary, and a 72-week regimen was recommended.

Discussion

This is the first study in which SVR, TR, and NR, in response to 48 weeks of peginterferon plus ribavirin treatment, were successfully distinguished in the early stage of treatment. This was possible by using new indices (rebound index: RI, and second rebound index: RI-2nd) calculated from early viral kinetics and the timing of when HCV RNA becomes undetectable. This allows for the TR group to be treated for 72 weeks, potentially raising the SVR rate.

In the treatment of genotype 1 CHC with peginterferon plus ribavirin, relapse occurs in about 30% of patients after the end of treatment [1, 2]. In LVR (late viral responders), in particular, the percentage of relapse is high (59%) after 48 weeks of treatment [7]. It is vital to reduce the relapse rate (TR rate) and raise the SVR rate. This requires (1) dose increase and (2) prolongation of the period of treatment. In Japanese patients, the dose of peginterferon must often be reduced because of the onset of such adverse events as neutropenia, thrombocytopenia, and malaise, and thus dose increase is not a feasible option. The dose of ribavirin must also be reduced in some patients due to ribavirin-induced anemia, and likewise, any increase in dose is not feasible [15]. Thus extending the duration of treatment to 72 weeks is considered necessary, and it becomes essential to distinguish the population for which 48-week treatment is adequate from the population for which 72-week treatment is necessary. In a previous study, HCV RNA-negativity was determined at weeks 12 and 24 [7]. SVR was noted in

Table 3 Kinetics of HCV RNA during the first 2 weeks of treatment by response (RVR, W8EVR, W12EVR, LVR, or NVR)

	RVR ($n = 26$)		W8 EVR ($n = 31$)		W12 EVR ($n = 31$)		LVR ($n = 24$)		NVR ($n = 29$)		P value[a]
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
HCV loads (×1000 IU/ml)											
Before	2102	(1636)[b]	2271	(1536)[c]	2259	(1744)	2625	(1278)	2390	(1501)	0.9172
24 h	143	(182)	186	(191)	134	(95)	563	(558)	523	(518)	0.0007
1 week	55	(66)	231	(213)	277	(325)	674	(503)	1206	(811)	<0.0001
2 week	8	(5)[b]	49	(86)[c]	119	(264)	361	(299)	615	(617)	<0.0001
Rebound index	0.4	(0.3)	2.3	(3.6)	2.4	(2.8)	1.5	(0.6)	3.1	(1.8)	<0.0001
Rebound index 2	0.1	(0.1)	0.5	(0.6)	0.7	(1.4)	0.7	(0.4)	1.3	(1.1)	<0.0001

RVR rapid viral response, *W8EVR* week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non viral response, *SD* standard deviation

[a] Kruskal Wallis Test

[b] $P < 0.001$ (hour 24 vs. week 2 in RVR)

[c] $P < 0.001$ (hour 24 vs. week 2 in W8 EVR)

18% even when treatment was continued to week 48 in patients who were HCV RNA-positive at week 12 but HCV RNA-negative at week 24 (LVR) [7]. If treatment is continued for 72 weeks, these patients will receive drugs unnecessarily for an extra 24 weeks. On the other hand, in this study, the SVR rate with W8EVR and W12EVR was 84% and 55%, respectively. The SVR rate for the overall EVR (W8EVR + W12EVR) was 69%, with a relapse rate of 31%. By treating these relapsed patients for 72 weeks, a higher SVR can be expected.

For more effective treatment with peginterferon plus ribavirin, indices besides the currently used index (the time to HCV RNA negativity) should be introduced and evaluated. In this study, we succeeded in differentiating the populations to be treated for 48 and 72 weeks more accurately by measuring the viral loads up to week 2 after the start of treatment and calculating two new indices (rebound index and second rebound index). Unrelated to ribavirin, lowering the dose of peginterferon at the early stage of treatment reduces the SVR rate [16]. In other words, the therapeutic effect of peginterferon, independent of that of ribavirin at the early stage of treatment, is expected to be responsible for SVR and EVR, which is believed to occur before ribavirin takes effect. Early viral kinetics were determined up to week 2, which are believed to express the therapeutic effect of peginterferon. The serum concentration of peginterferon alpha 2b peaks after 24 h, followed by a gradual decline [17, 18]. The viral load is therefore reduced by 24 h but increases in week 1 [19, 20]. A large dose of peginterferon at each administration results in a marked reduction in the viral load at 24 h but the viral load increases in week 1 regardless of the dose. In the responder group, the viral load continues to decline each week thereafter [20]. This trend is also seen with peginterferon monotherapy [17]. On the other hand, in the SVR group, in particular the RVR group, it was noted that a number of patients did not experience an increase in the viral load at week 1. As shown in Fig. 1, in SVR, the viral load does not increase at week 1, while a return of viral loads is seen in TR and NR. The viral loads of SVR and TR were lower than that of NR at week 2. The viral load in week 1 divided by the viral load at 24 h was therefore defined as the rebound index (RI). The RI of SVR is 0.8 (less than 1.0), which is less than that for TR or NR. Among the RI of RVR, W8EVR, W12EVR, LVR, and NVR, only that of RVR was below 1.0. Among the 26 RVR patients, 24 (92%) exhibited RI-A (RI: $\leq$ 1.0) without a rise in week 1 (Fig. 3). The SVR rate with RI-A was 90%. It was believed that this group (RI-A, RI: $\leq$ 1.0) was composed of high responders to peginterferon. Because no decline in the viral load is noted in non-responders after week 2 [20], the viral load at week 2 divided by the viral load at 24 h was defined as the second rebound index (RI-2nd). Compared with SVR and TR, RI-2nd with NR was high. The patients with high RI-2nd were suspected to be poor responders or non-responders to peginterferon. RI-2nd of those other than NVR was below 0.7; and therefore 0.7 was adopted as the reference value for RI-2nd.

Based on the results of our study (Fig. 3), the SVR rate was very high (about 90%) in 3 areas of SVR: RI-A (RI: $\leq$ 1.0), RVR, and RI-B (RI-2: $<$ 0.7) and W8EVR. These are believed to represent the areas for which 48-week treatment is recommended. About 60% of the 112 patients who became HCV RNA-negative within 24 weeks were in this area, and the SVR rate of the remaining 40% was low (25%) while the TR rate was high (75%). It was therefore thought that 72-week treatment is needed for these patients.

In peginterferon and ribavirin treatment, the status of EVR is important. When HCV RNA-negativity is not achieved by week 12, the SVR rate becomes very low [10].

	RVR N=26	W8EVR N=31	W12EVR N=36	LVR N=26	NVR N=29	Total N=148
RI>1.0, RI-2nd≥0.7 RI-C, N=52		△△△	△△△△ ○△△△△△	△△△△△△ ○△△△△△	□□□ □□□□□□ □□□□□□ □□□□□□ □□□□□□	SVR:3.9% TR:44.2% NR:51.9%
RI>1.0, RI-2nd<0.7 RI-B, N=45	○△	○○△ ○○○○○○ ○○○○○○	△△ ○△△△△△ ○○○○○○	△△△△△△ ○○○△△△	□□	SVR:55.6% TR:30.0% NR:4.4%
RI≤1.0 RI-A, N=51	○○○○○○ ○○○○○○ ○○○○○○ ○○○○○○	△ ○○○○○○ ○○○○○○	○○○△△△ ○○○○○○	○△		SVR:90.2% TR:9.8% NR:0.0%
Total N=148	SVR:96.2% TR:3.8% NR:0.0%	SVR:83.9% TR:16.1% NR:0.0%	SVR:47.2% TR:52.8% NR:0.0%	SVR:19.2% TR:80.8% NR:0.0%	SVR:0.0% TR:0.0% NR:100%	SVR:49.3% TR:31.1% NR:19.6%

Fig. 3 SVR, TR, and NR by RI and RI-2nd as well as RVR, W8EVR, W12EVR, LVR, and NVR. *SVR* white circle, *TR* white triangle, *NR* white square, *SVR* sustained virologic response, *TR* transient response, *NR* non-response, *RI* rebound index, *RI-2nd* rebound index second, *RVR* rapid viral response, *W8EVR* week 8 early viral response, *W12EVR* week 12 early viral response, *LVR* late viral response, *NVR* non-viral response

In our study, SVR was low (below 20%) among the LVR who became negative for HCV RNA between weeks 12 and 24. Mangia et al. reported that to raise the SVR rate, treatment for 48 weeks is needed if HCV RNA becomes negative at week 8, while treatment for 72 weeks is needed if HCV RNA negativity is observed at week 12 [21]. In our study, the SVR rate with RVR was 96% and was also very high (84%) with W8EVR achieving HCV RNA negativity between 5 and 8 weeks. On the other hand, the SVR rate was low (55%) in patients who became HCV RNA-negative between weeks 9 and 12. These findings suggested that in treating Japanese patients with CHC with peginterferon plus ribavirin for 48 weeks, EVR should be qualified at week 8 rather than at week 12. Therefore, for evaluation, EVR patients who became HCV RNA-negative by week 8 were classified as W8EVR and those who became HCV RNA-negative by week 12 were classified as W12EVR. Early viral kinetics of both W8EVR and W12EVR indicated a rebound at week 1 but the viral load of W8EVR at week 2 was significantly lower than that at 24 h. On the other hand, the reduction in the viral load of W12EVR at week 2 was not significant when compared against that at 24 h. A significant reduction in the viral load was observed with RVR and W8EVR at week 2 compared to that at 24 h, and the SVR rates were correspondingly very high. It was believed that the reduction in the viral load at week 2 is important.

Real-time PCR assay is now commonly used and is more sensitive for detecting serum HCV than the COBAS Amplicor HCV Monitor assay. Its use may have allowed viral detection for a longer period of time, possibly resulting in the number of RVR and EVR patients being reduced while the SVR rate in RVR and EVR patients was increased. Examination of the SVR rate by the timing of HCV RNA negativity using real time PCR assay will be necessary in the future.

Reduction in the duration of treatment is being investigated for the good responders to peginterferon plus ribavirin treatment. In RVR patients who achieve HCV RNA-negativity at week 4, the SVR rate is reported to be 89% when treatment is continued for 24 weeks [11]. In this study, all patients in the RI-A (RI ≤ 1.0) and RVR area became SVR; thus they were believed to be extremely good responders. A more detailed investigation with a larger number of subjects is necessary to elucidate the question of a reduction in the duration of therapy.

The explanation of early viral kinetics by SVR, TR, and NR is highly complex and is impractical in clinical use. In this study, RI and RI-2nd calculated from early viral kinetics were used. It is believed that the simplified RI and RI-2nd are effective indices to determine the therapeutic efficacy of peginterferon therapy alone. By combining these two new indices and the indices for therapeutic efficacy of peginterferon plus ribavirin (RVR, W8EVR, W12EVR and LVR), SVR was distinguished from TR during treatment. With the aid of these indices, it is believed that a more effective peginterferon plus ribavirin treatment will be possible. We used these new indices in this study, and the measurement of HCV RNA levels was conducted using the COBAS Amplicor HCV Monitor assay. Since the range of detection of HCV is narrow with this assay, there were many patients with pretreatment HCV levels above the limit of detection. The timing of HCV RNA negativity and examination based on the HCV levels at week 1 and week 2 needs to be conducted using real time PCR assay in future studies. A larger scale study should be conducted to examine the duration of treatment for patients who are on reduced doses of peginterferon and ribavirin.

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The loss of HBeAg without precore mutation results in lower HBV DNA levels and ALT levels in chronic hepatitis B virus infection

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Abstract
Background The aim of this study was to investigate the correlation between precore (PC)/basal core promoter (BCP) mutations and the viral loads or activity of hepatitis in patients with chronic hepatitis B virus (HBV) infection.
Methods HBV genotypes, PC mutations, BCP mutations, HBV DNA levels, and serological markers of HBV were analyzed in all the patients with chronic HBV infection seen in Fujita Health University Hospital from June 2004 to November 2008 ($n = 215$).
Results HBV genotype was C in 169 patients, B in 16, A in 3, F in 1, and unclassifiable in 5. Among the patients with genotype C, the prevalence of PC wild type was significantly lower in hepatitis B envelope antigen (HBeAg)(−) patients than in HBeAg(+) patients (9.5% versus 49.0%, $P < 0.0001$). Among HBeAg(−) patients, the patients with PC wild type had significantly lower serum viral loads and alanine aminotransferase (ALT) levels compared with those with PC mutant ($P < 0.001$). Among HBeAg(−) patients, the patients with genotype B had lower serum viral loads compared with those with genotype C (3.6 ± 0.9 versus 4.6 ± 1.6, $P < 0.05$), and the prevalence of BCP wild type was significantly higher in those with genotype B than in those with genotype C (58.3% versus 10.8%, $P < 0.05$).
Conclusions Among HBeAg(−) patients with genotype C, the patients with PC wild type had significantly lower viral loads and ALT levels than those with PC mutant. This suggests that the patients with PC wild type may have better prognosis than those with PC mutant among HBeAg(−) patients with genotype C.

Keywords Precore mutation · Basal core promoter mutation · Hepatitis B virus · HBV genotype

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Introduction

Hepatitis B virus (HBV) is one of the most frequent and important causes of chronic viral hepatitis worldwide. It is estimated that approximately 2 billion people have had contact with the virus and more than 400 million people are chronic carriers [1, 2]. Chronic HBV infection is associated with a wide range of clinical manifestation, from an asymptomatic carrier to chronic liver disease, which may lead to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).

Seroconversion from hepatitis B envelope antigen (HBeAg) to its antibody (anti-HBe), either spontaneous or after antiviral therapy, is usually accompanied by a decrease in viral replication and remission of liver disease [3, 4]. However, viral replication and hepatic inflammation persist in about 10% of patients after seroconversion [5, 6]. The exact mechanism of seroconversion has not been fully elucidated, but several mutations in the HBV genome have been reported to be associated with the phenomenon. The mutation of A to G at nucleotide 1896 in the precore (PC) region (G1896A), which converts codon 28 for tryptophan to a stop codon, is associated with the loss of HBeAg [7]. The dual mutation (A1762T and G1764A) in basal core promoter (BCP) region has been shown to reduce the synthesis of HBeAg by suppressing the transcription of

precore mRNA [8, 9]. PC/BCP mutations have been reported to be associated with fulminant hepatitis [10, 11]. Recent study reported that the presence of BCP mutation was an independent risk factor for liver cirrhosis, as well as old age and genotype C infection [12]. However, the relationship of the mutations in PC and BCP region with serum HBV DNA levels, and severity of liver disease is still not fully elucidated.

HBV is classified into eight genotypes designated A to H, based on an intergroup divergence of 8% or more in the complete nucleotide sequence [13, 14], and the distribution of HBV genotypes is geographically restricted [15, 16]. Resent studies suggested that HBV genotypes may be related to the rate of recovery from acute HBV infection and the progression of liver disease during chronic HBV infection [17–20]. Genotype C and B are predominant genotypes in Japan, and it has been reported that HBV genotype C is associated with more severe liver diseases than is genotype B [17, 19]. Several studies have shown that genotype C has a higher frequency of the dual mutation in the BCP region than genotype B [5, 18, 19]. However, the prevalence of the mutations in PC and BCP region of these two genotypes with different HBeAg status and its correlation with viral load are not clearly reported.

The aim of this study was to determine whether the mutations in PC and BCP region gave useful information on determining the management of patients with chronic HBV infection. The correlation between these mutations and the viral loads or activities of hepatitis was investigated.

Patients and methods

Patients

This is a retrospective study using stored sera obtained from all the patients with chronic HBV infection seen in Fujita Health University Hospital from June 2004 to November 2008. Chronic HBV infection was defined as persistent seropositivity for HBV surface antigen (HBsAg) for at least 6 months before enrollment. Patients diagnosed with acute hepatitis were excluded. Patients on antiviral therapy were also excluded because of the effects of treatment on serum HBV DNA levels and the possibility that antiviral therapy might have an impact on PC and BCP mutations. All patients were negative for antibodies to hepatitis C virus (HCV), and had no serological markers suggestive of autoimmune disease. Demographic, clinical, and laboratory data were collected during clinic visits. Laboratory results on hepatitis B markers (HBsAg, HBeAg, anti-HBe), liver chemistry including alanine aminotransferase (ALT) values, and platelet count were also recorded. The blood samples were collected during clinic visits and the sera were stored at −80°C as aliquots until use. This study adhered to the guidelines of the 1975 Declaration of Helsinki, and all patients gave informed consent.

HBV genotyping

HBV genotype was determined by restriction fragment length polymorphism (RFLP) method on S-gene sequence amplified by polymerase chain reaction (PCR) with nested primers. The genomic segment between nt256 and nt796 in the S region was amplified by PCR. After a subsequent incubation with Hinf1 and Tsp509I, genotype was determined from the RFLP patterns on agarose electrophoresis [21, 22].

Quantification of serum HBV DNA levels

Serum HBV DNA level was quantified by PCR assay using an Amplicor HBV monitor kit (Roche Diagnostics, Tokyo, Japan) which had a quantitative range of 2.6–7.6 log copies/ml [23].

Detection of PC and BCP mutations

The stop codon mutation in the PC region (A1896) was detected with an enzyme-linked mini-sequence assay kit (Smitest HBV Pre-C ELMA; Roche Diagnostics, Tokyo, Japan). In principle, G1896 in the wild-type HBV and A1896 in the mutants were determined by mini-sequence reactions using labeled nucleotides that are complementary to either the wild type or mutant. The results were expressed as percentage mutation rate according to the definition by Aritomi et al. [11]. The sample was judged positive for the PC mutation when the mutation rate attained 100% in the present study, and judged mixture status of mutant and wild-type sequence in PC region when the mutation rate was in the range 10–90%. The dual mutation in BCP region was detected with an enzyme-linked specific probe assay (Smitest HBV core promoter mutation detection kit; Roche Diagnostics, Tokyo, Japan) [11]. This kit detects T1762/G1764 or A1762/T1764 by PCR with primers specific for either the wild type or mutant. The results were recorded in three categories: wild, mixed, and mutant types. The detection limits of these kits are both 1,000 copies/ml.

Statistical analysis

Results are expressed as mean ± standard deviation. Data were analyzed using StatView version 5.0 software

package (SAS, Cary, NC). Statistical analyses were performed using χ^2 and Fisher's extract test for categorical variables. Mann–Whitney U test was used for comparison of continuously distributed variables between two independent groups. Results were considered statistically significant at $P < 0.05$.

Results

Baseline characteristics and HBV genotypes

A total of 215 patients were studied. The study population included 154 (71.6%) men and 61 (28.4%) women; mean age was 48.5 ± 13.7 years (range 18–82 years). All the patients were ethnically Japanese. Clinical diagnoses of the patients were as follows: 67 patients were asymptomatic carriers, 97 had chronic hepatitis, 28 had cirrhosis, and 18 had HCC. At presentation, 73 (34.0%) patients were HBeAg positive.

Serum HBV DNA was detected in 100% (73/73) of HBeAg-positive patients and in 85.2% (121/142) of HBeAg-negative patients after nested PCR. The overall HBV detection rate was 90.2% (194/215). Four HBV genotypes were found: 169 genotype C (87.1%), 16 genotype B (8.2%), 3 genotype A (1.6%), and 1 genotype F (0.5%). In five samples (2.6%), genotype could not be determined. We focused the study on 185 patients with genotype C or genotype B.

The baseline characteristics of the 185 patients with HBV genotype C and B are summarized in Table 1. Genotype B patients had significantly lower ALT values ($P < 0.05$), lower HBV DNA levels ($P < 0.05$), and lower prevalence of HBeAg ($P < 0.05$) compared with genotype C patients.

Table 1 Baseline characteristics of patients with HBV genotype C and genotype B

	Genotype C ($n = 169$)	Genotype B ($n = 16$)	P value
Mean age (years)	46.9 ± 13.1	52.2 ± 15.6	NS
Gender (male:female)	118:51	10:04	NS
Clinical diagnosis			
Asymptomatic carrier	44 (26.1%)	10 (62.5%)	
Chronic hepatitis	82 (48.5%)	5 (31.2%)	
Liver cirrhosis	23 (13.6%)	1 (6.3%)	
Hepatocellular carcinoma	20 (11.8%)	0 (0%)	
ALT (IU/L)	76 ± 101	26 ± 21	<0.05
Platelet count (K/mm^3)	162 ± 61	192 ± 41	NS
HBeAg positive (%)	41.4	0	<0.05
HBV DNA (log copies/ml)	5.6 ± 1.9	3.5 ± 0.9	<0.05

NS not significant

Comparison between HBeAg-positive patients and HBeAg-negative patients

Among the patients with genotype C, HBeAg-positive patients had significantly higher HBV DNA levels ($P < 0.001$) and higher ALT levels ($P < 0.05$) than HBeAg-negative patients (Table 2). The mean level of serum HBV DNA levels ($P < 0.001$) and ALT levels ($P < 0.05$) were significantly higher in HBeAg-positive patients with genotype C than in HBeAg-negative patients with genotype B. Among HBeAg-negative patients, HBV DNA levels were significantly higher in genotype C patients than in genotype B patients ($P < 0.05$).

Prevalence of PC mutation in patients with HBV genotype C

Compared with HBeAg-positive patients, HBeAg-negative patients were significantly less likely to have PC wild type in patients with HBV genotype C ($P < 0.0001$) (Table 3). HBeAg-negative patients with PC wild type had significantly lower HBV DNA levels ($P < 0.05$) and ALT levels

Table 2 Comparison between HBeAg(+) patients and HBeAg(−) patients

	HBV DNA (log copies/ml)	ALT (IU/L)	Platelet count (K/mm^3)
Genotype C			
HBeAg(+) (n=70)	7.1 ± 0.9	111 ± 139	163 ± 64
HBeAg(−) (n=99)	4.5 ± 1.6	51 ± 53	162 ± 60
Genotype B			
HBeAg(+) (n=0)	–	–	–
HBeAg(−) (n=16)	3.5 ± 0.9	26 ± 21	192 ± 41

* $P<0.05$ ** $P<0.001$

Table 3 Prevalence of precore mutation in patients with HBV genotype C

	Prevalence (%)	HBV DNA (log copies/ml)	ALT (IU/L)	Platelet count (K/mm^3)
HBeAg(+) PC-Wild (n=36)	51.47	7.3 ± 0.6	99 ± 135	168 ± 55
HBeAg(+) PC-Mix (n=30)	42.9	7.0 ± 1.0	131 ± 150	151 ± 61
HBeAg(+) PC-Mutant (n=4)	5.7	5.6 ± 2.8	71 ± 58	201 ± 137
HBeAg(−) PC-Wild (n=11)	11.1	3.3 ± 0.9	21 ± 10	194 ± 79
HBeAg(−) PC-Mix (n=26)	26.3	4.9 ± 1.7	54 ± 53	143 ± 53
HBeAg(−) PC-Mutant (n=59)	59.6	4.6 ± 1.4	57 ± 56	163 ± 58
HBeAg(−) PC-UD (n=1)	3.0	2.7 ± 0.2	27 ± 23	188 ± 47

* $P<0.05$ ** $P<0.001$ *** $P<0.0001$

PC precore, *UD* undetectable

Table 4 Prevalence of basal core promoter mutation in patients with HBV genotype C

	Prevalence (%)	HBV DNA (log copies/ml)	ALT (IU/L)	Platelet count (K/mm^3)
HBeAg(+) CP-Wild ($n = 17$)	24.3	7.5 ± 0.4	122 ± 182	186 ± 62
HBeAg(+) CP-Mix ($n = 11$)	15.7	6.9 ± 1.1	116 ± 121	177 ± 49
HBeAg(+) CP-Mutant ($n = 42$)	60	7.0 ± 1.1	105 ± 126	149 ± 66
HBeAg(−) BCP-Wild ($n = 13$)	13.1	3.7 ± 1.4	39 ± 58	182 ± 47
HBeAg(−) BCP-Mix ($n = 2$)	2.0	3.5 ± 0.9	26 ± 1	155 ± 27
HBeAg(−) BCP-Mutant ($n = 82$)	82.9	4.7 ± 1.6	55 ± 53	157 ± 62
HBeAg(−) BCP-UD ($n = 2$)	2.0	2.6 ± 0.0	26 ± 1	216 ± 30

BCP basal core promoter, *UD* undetectable

Table 5 Prevalence of precore and basal core promoter mutations in patients with HBV genotype B

	Prevalence (%)	HBV DNA (log copies/ml)	ALT (IU/L)	Platelet count (K/mm^3)
HBeAg(+) ($n = 0$)	–	–	–	–
HBeAg(−) PC-Wild ($n = 3$)	18.8	3.9 ± 0.8	31 ± 20	192 ± 13
HBeAg(−) PC-Mix ($n = 3$)	18.8	3.3 ± 0.6	33 ± 16	168 ± 47
HBeAg(−) PC-Mutant ($n = 10$)	62.4	3.7 ± 1.0	23 ± 22	199 ± 42
HBeAg(−) BCP-Wild ($n = 11$)	68.7	3.7 ± 0.9	27 ± 23	203 ± 41
HBeAg(−) BCP-Mix ($n = 0$)	–	–	–	–
HBeAg(−) BCP-Mutant ($n = 2$)	12.5	3.5 ± 0.6	18 ± 2	184 ± 7
HBeAg(−) BCP-UD ($n = 3$)	18.8	3.0 ± 0.6	31 ± 20	157 ± 7

PC precore, *BCP* basal core promoter, *UD* undetectable

($P < 0.001$) compared with those with PC mutant. There were no significant differences in the mean level of platelet count between HBeAg-positive patients and HBeAg-negative patients, and among the patients with different patterns of PC sequences.

Prevalence of BCP mutation in patients with HBV genotype C

There was no significant difference in prevalence of BCP mutants between HBeAg-positive patients and HBeAg-negative patients with HBV genotype C (Table 4). There were no significant differences in serum HBV DNA levels, ALT levels, and platelet counts among the patients with different patterns of BCP sequences.

Prevalence of PC and BCP mutations in patients with HBV genotype B

All patients with HBV genotype B were HBeAg negative (Table 5). Among HBeAg-negative patients, genotype B patients were significantly more likely to have BCP wild type than were genotype C patients (68.7% versus 13.1%, $P < 0.001$), while there was no significant difference in prevalence of PC mutants between HBe-negative patients with HBV genotype B and those with HBV genotype C (62.4% versus 59.6%). There were no significant differences in the mean levels of serum HBV DNA, ALT, and platelet counts among the patients with different patterns of PC and BCP sequences. In comparison with genotype C patients, the patients with PC mutant tended to have lower HBV DNA levels and ALT levels in genotype B patients ($P = 0.0753$ and $P = 0.0605$).

Discussion

The present study demonstrated that, among HBeAg-negative patients with genotype C, the patients with PC wild type had significantly lower HBV DNA levels and ALT levels than those with PC mutant. Only a few studies have described the correlation of PC mutations with HBV DNA levels and ALT levels in HBeAg-negative patients. Misawa et al. [24] reported that PC and BCP mutations were significantly more frequent in 18 patients with positive HBV DNA than in 6 patients with negative HBV DNA among HBeAg-negative patients. The results of present study are consistent with their findings, although the present study found no correlation of BCP mutations with HBV DNA levels. On the other hand, Yoo et al. [25] found no correlation of PC mutation with HBV DNA levels or disease severity in HBeAg-negative patients with genotype C. The discrepancy between the present study and the study of Yoo et al. may be due to the difference of the detection method of HBV DNA; Yoo et al. used branched DNA assay which is less sensitive than Amplicor HBV monitor used in the present study.